Allelopathic alkaloids of an invasive shrub and their effect on the growth of ectomycorrhizal fungi

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Abstract

One mechanism of invasive species success is the production of allelopathic chemicals that negatively affect native competitors. A highly invasive shrub, *Cytisus scoparius*, impedes Douglas-fir tree establishment in clearcuts, even years after its removal. This impediment may be from the allelopathic alkaloids of *C. scoparius* that could indirectly hinder Douglas-fir by inhibiting their mutualistic ectomycorrhizal fungi (EMF). I extracted and quantified alkaloids from *C. scoparius* tissue for use in a laboratory bioassay. I then tested if and how these alkaloids affected EMF growth. In a second assay, I tested the effects of three concentrations of pure sparteine, the primary alkaloid in *C. scoparius*, on fungal growth. Sparteine was the only alkaloid recovered from the extraction which yielded 0.32 mg sparteine/g fresh weight, a lower concentration than previously reported values. Both the crude extract and pure sparteine significantly affected fungal growth, but only sparteine produced a species-specific response. Growth was inhibited by increasing sparteine concentrations, and most species were inhibited at 1.4 mM, the concentration found in *C. scoparius*. One common EMF, *Wilcoxina mikolae,* was unaffected by sparteine while others, like S*uillus caerulescens* and *Cenoccoccum geophilum,* were more sensitive and stopped growing entirely at 5 to 10 mM. These results suggest that the alkaloids of *C. scoparius* may seriously hinder EMF, and indirectly Douglas-fir, contributing to the competitive dominance of the invasive shrub.

Keywords: Douglas-fir, *Cytisus scoparius*, allelopathy, ectomycorrhizal fungi, alkaloids, sparteine

Introduction

Invasive plants alter ecosystems and reduce biodiversity (Vitousek and Walker 1989, Simberloff 2005) yet many mechanisms underlying their success remain elusive (Lodge 1993). Invasive species reduce native plant population sizes and alter community structure by outcompeting native organisms through alteration of their biotic or abiotic environment (Hejda et al. 2009, Rejmánek et al. 1995,Williamson 1996, Parker et al. 1999, Ehrenfeld 2003, Lambertini et al. 2011). Some invasive plants alter their environment through allelopathy - the production of chemicals that adversely affect other species (Callaway and Ridenour 2004, Vivanco et al. 2004, Pollock et al. 2008, Jarchow and Cook 2009, Lankau et al. 2009). Secondary metabolites of plants include tannins, phenols, gluconates, and alkaloids and probably serve primarily as defensive agents against enemy attack by pathogens and herbivores (Bennett and Wallsgrove 1994, de La Vega et al. 1996) but can also have negative effects on plant competitors (Callaway and Aschehoug 2000, Prati and Bossdorf 2003)

In the introduced ranges of invasive plants, these defense chemicals may also have particularly strong effects on co-occurring native species due to a lack of evolutionary history between these species. Native species that have not encountered the secondary metabolites of introduced species may have evolved fewer mechanisms to counter their toxicity (Callaway and Aschehoug 2000). For example, the invasion success of the European weed, *Centaurea maculosa*, in North America, is in part attributed to the allelopathic phenol, (±)-catechin. The addition of both *C. maculosa* and (±)-catechin

suppressed the growth of plants native to North America while their European congeners were generally less affected (He et al. 2008).

Consequences of allelopathy are not limited to plants, but can also impact fungi (Hagan et al. 2013, Schreiner and Koide 1993, Cipollini et al. 2012). Invasive plant allelochemicals have also been shown to prevent the growth of mutualistic arbuscular mycorrhizal fungi (Stinson et al. 2006, Wolfe et al. 2008). This is significant because mycorrhizal symbioses are ubiquitous, an estimated 80% of terrestrial plants, and 100% of conifers, form mycorrhizal associations (Wang 2006). Ectomycorrhizal fungi (EMF), a group of mycorrhizal fungi, form a sheathing mantle on plant roots and extend hyphae out into the soil to scavenge otherwise unavailable forms of phosphorus and nitrogen for plants, and in return, receive carbohydrates directly from roots (Smith and Read 1997). Other services of EMF include drought resistance and protection against pathogens (Borowicz 2001). Suppression of ectomycorrhizal fungi, through allelopathy, can therefore have indirect consequences on the performance of their plant hosts.

Allelochemicals of invaders may disrupt the mycorrhizal mutualism of native plants and fungi by inhibiting fungal survival or suppressing the ability of fungi to colonize plant roots (Hagan et al. 2013, Bever et al. 2009). *Imperata cylindrica*, an especially problematic invasive in the southeast US, releases phenolic acids that inhibit mycorrhizal colonization of native plants, which decreases native plant biomass (Hagan et al. 2013). Allelopathic chemicals can also impede the growth of mycorrhizal fungi. Invasive *Alliaria petiolata*, for example, is thought to be such a successful invader because of its allelopathic glucosinolates (Wolfe et al. 2008). An *in situ* study by Canor

et al. (2010) found reduced hyphal abundance in soil invaded by invasive *A. petiolata* compared to uninvaded soils, most likely due to these allelochemicals.

If allelopathic chemicals of invasive plants remain in the soil after invader removal, negative effects on mycorrhizal mutualisms can persist as a 'soil legacy effect' (Inderjit et al. 2006, Pollock et al. 2008). Soil legacies lead to continued inhibition of mycorrhizae, as is the case with *A. petiolata* and its glucosinolate products (Schreiner and Koide 1993, Stinson et al. 2006, Wolfe et al. 2008). Native hardwood trees grown in soil previously conditioned with *Alliaria* hosted less arbuscular mycorrhizae and were smaller than trees grown in uninvaded soil (Stinson et al. 2006). Considering the ubiquity and importance of mycorrhizae in plant performance, continued disruption of the mutualism could have many long-term effects on the restoration of plant species highly dependent on their fungal symbionts.

Cytisus scoparius is a large shrub native to western Europe that has invaded temperate areas worldwide (Mobley 1954, Gilkey 1957, Peterson and Prasad 1998, Isaacson 2000, Smith et al. 2000). In the Pacific Northwest region of the US it is very problematic, both ecological and economically (Hulting et al. 2008, Matsen 2011). In Oregon State alone, *C. scoparius* costs approximately $40 million a year in lost timber revenue and removal expenses (Hulting et al. 2008). Even after *C. scoparius* has been removed, restoration of Douglas-fir forests often fail in invaded areas. This may be because *C. scoparius* is rich in quinolizidine alkaloids that inhibit enemy attack by bacterial and fungal pathogens (Wink et al. 1982, Küçükboyacı et al. 2012, Bernal-Alcocer et al. 2005, Wink 1984, de La Vega et al. 1996), and could also have allelopathic effects on

EMF important to Douglas-fir seedlings. *C. scoparius* synthesizes numerous alkaloids but the primary constituent is sparteine (Wink 1981): a lupanine derivative and sodium ion channel blocker that inhibits the formation of an action potential for ATP synthase (Polya et al. 2013).

The soil legacy effects of *C.scoparius* in Douglas-fir forests may be in part due to the persistence of sparteine in soil following *C. scoparius* removal. Grove et al. (2012) found that Douglas-fir seedlings grown in soil invaded by *C.scoparius* were smaller than those grown in uninvaded soil. Seedlings grown in invaded soil also had less EMF colonization. I hypothesize that sparteine leaches from *C. scoparius* tissue into the soil, where it inhibits development of Douglas-fir EMF communities, hindering Douglas-fir establishment and growth.

Sparteine has known antimicrobial properties against both bacterial and fungal pathogens (Wink 1984, Wippich et al. 1985) but there have been no prior studies looking at the effects of quinolizidine alkaloids on mycorrhizal fungi. Previous studies have shown that these alkaloids, while antimicrobial, do not affect all microbes to the same extent. Fungal pathogens exposed to quinolizidine alkaloids have highly species-specific growth responses as some species are more tolerant of the metabolites than others (Bernal-Alcocer et al. 2005, Zamora-Natera et al. 2005). Fungal tolerance of plant secondary compounds can reflect the ability to enzymatically decompose allelochemicals into less harmful compounds (Ito et al. 2004, Sandrock et

al. 1998) or an innately resistant physiology (Défago et al. 1983). It follows that Douglas-fir EMF might also react to *Cytisus* alkaloids in such a species-specific manner.

The purpose of this study was to determine to what extent *C. scoparius* alkaloids affect growth of fungi associated with Douglas-fir. I measured the growth response of both EMF and non-EMF species cultured from Douglas-fir seedlings to a crude alkaloid extract from *C. scoparius* tissue and varying levels pure sparteine. Using a crude extract from *C. scoparius* allowed us to capture the allelopathic effects of all its alkaloid compounds together, as other alkaloids may also have fungicidal properties. Comparing responses to the crude extract and pure sparteine allowed us to test whether sparteine is the primary alkaloid responsible for the suppression of fungal growth. I predicted that 1) increasing concentrations of alkaloids would be inversely proportional to EMF growth and 2) fungal growth response will be highly species-specific.

Methods

Study Species: Four fungal species were isolated from the roots of Douglas-fir trees (methods described below) for use in bioassays (Table 1). I additionally obtained cultures of three EMF species commonly associated with Douglas-fir in the Pacific Northwest, but which I was unable to isolate from our roots. These include: *Wilcoxina mikolae var. tetraspora, Cenococcum geophilum*, and *Suillus caerulescens* (Table 1). I purchased these from the CBS-KNAW Fungal biodiversity Centre in Utrecht, the Netherlands.

The two mycorrhizal fungi isolated were *Tomentella lateritia* and *Oidiodendron periconiodes*. *T. lateritia* (Thelephoraceae) is a widespread ectomycorrhizal fungi and is commonly associated with Douglas-fir (Bruns et al.1998, Larsen 1967). *Oidiodendron periconioides* (Myxotrichaceae) has been found as an ericoid mycorrhiza and soil fungus (Currah et al. 1993) but can also act as a decomposer as it degrades tannic acid and pectin (Rice et al. 2005).

The non-mycorrhizal species that were isolated are both members of the cosmopolitan soil fungus genus *Lecythophora* (Perdomo et al. 2013). *Lecythophora mutabilis* (Coniochaetaceae) is a soil-dwelling fungus, root endophyte of *Betula* and *Abies* (Kernaghan et al. 2011), and occasionally causes soft-rot wood decay (Rayner and Boddy 1988). It is prevalent in North America, Europe, and Australia (Williams and Spooner 1991). The functional role of *Lecythophora fasciculata* (Coniochaetaceae) is still unknown but many closely related species in the genus are root endophytes (Chen et al. 2011)

Cenococcum geophilum (Gloniaceae) is a widespread ectomycorrhizal fungus that forms only sterile mycelia (Trappe 1962). It forms a black hyphal sheath around roots making it an easily recognizable EMF. *C. geophilum* is common in all stages of forest succession (Visser 1995) and frequently forms mutualisms with Douglas-fir (Goodman et al. 1988). Its wide distribution stems from its ability to tolerate many environmental stressors such as drought (Pigott 1982).

Suillus caerulescens (Suillaceae) is an EMF that associates with Douglas-fir in the Pacific Northwest. It is present throughout all stages of forest succession and is very sensitive to drought stress (Norvell et al. 2004; Coleman et al.1989). *Wilcoxina mikolae var. tetraspora* (Pyronemataceae) is an ectomycorrhizal fungus that forms associations with a variety of plants including *P. menziesii, Abies, Pinus, Lithocarpus*, and *Arbutus* (Yu and Egger 2001). It produces chlamydospores that persist in the soil for years (Nguyen et al. 2012) and is very tolerant of nutrient poor soil and heavy metal contamination (Mikola 1988).

Fungal isolation: Fungi were isolated from roots of *P. menziesii* seedlings grown in the UCSC greenhouses for previous research done by Grove (2014). Local Douglas-fir seeds were obtained from Silvaseed (Roy, Washington) and grown in soil collected from uninvaded forests in the Joint Base Lewis McChord (JBLM) military base, approximately 10 miles south of Tacoma, WA. I grew seedlings in 164 ml cone-tainers (Stuewe and Sons, Tangent OR) for 3 years before harvesting trees and washing roots with DI water. I randomly subsampled and selected 7, 5mm long root tips from each of 9 different trees to select for a wide variety of fungi as individual trees can host different EMF. I plated root tips on chloramphenicol-malt extract agar [20g malt extract, 15 g agar, 0.200 g chloramphenicol, 1 L deionized water] and grew cultures at room temperature. To isolate individual cultures, fungi were later transferred to

potato-dextrose agar plates [39 g potato-dextrose agar (Difco), 1L DI H2O] until sequencing.

As many fungi are difficult to identify visually, I identified species with Sanger DNA sequencing. I extracted DNA with the DNeasy Mini Plant Kit (Qiagen) and followed standard protocols combined with methods from Griffin et al. (2002). All steps were the same as those provided by Qiagen except that instead of bead-beating tissue, I froze 5 mm^2 pieces of hyphae on dry ice for one minute to lyse cells and then homogenized them with sterile, plastic pestles. Additionally, samples went through 7 freeze-thaw cycles that consisted of one minute on ice followed by one minute in a 65°C water bath and a final 30 min incubation in the water bath. I amplified a fungal specific ITS region through PCR with ITS-1F and ITS-4 primers and Green GoTaq master mix (Promega). The PCR product was cleaned with ExoSap-It (USB Products) and sequenced by the UC Berkeley Sequencing Facility (Berkeley, CA, USA). I identified sequences with Geneius software (Biomatters Ltd) and the NCBI GenBank database according to Grove et al. (2012). Cultures unidentifiable to species using BLAST were identified with the User-friendly Nordic ITS Ectomycorrhiza Database (UNITE), a database specifically created for mycorrhizal fungi.

Crude Alkaloid Extraction: *C. scoparius* was grown from seed in the UCSC greenhouses in 164 ml cone-tainers. Both seed and soil were from invaded forests in JBLM. After several months, seedlings were transplanted to larger containers filled with potting soil

and grew for four months prior to alkaloid extraction. I blended 102 g of fresh *C. scoparius* stem and leaf tissue with 20 ml of DI water and separated alkaloids with an acid-base extraction using 0.1 M HCl and 0.1 M NaOH. Supernatant was filtered with a buchner funnel and alkaloids separated with DMSO. Alkaloids were dried with anhydrous Na_2SO_4 and the remaining water evaporated.

Extract analysis was performed by HPLC-mass spectronomy with a 1525 binary HPLC pump (Waters), autosampler, and a 2998 photodiode array (Waters). Sample injection size was 10 µl. I used an Accucore-150-C18 reverse-phase column (ThermoScientific), 50x4.6mm, for HPLC separation. The sample was eluted for 5 minutes on a 10-100% gradient. Chromatography was carried out with acetonitrile in water and 0.1% formic acid as solvents at room temperature. The sample was then analyzed by electron mass spectroscopy with a MicroMass quadrupole mass spectrometer (Waters). I compared peaks on the MS output to molecular masses of known *C. scoparius* alkaloids for compound identification.

Bioassays: I used fungi in two bioassays, one with crude alkaloid extract and the other pure sparteine, to ascertain the effects of *C. scoparius* alkaloids on EMF growth. For the crude extract assay, I filled each well of a 12-well plate with 1 ml of sterile potato dextrose broth (PDB) [24g potato-dextrose (Difco), 1L DI H_2O]. Half of the wells received plain PD broth and the other a broth containing crude alkaloid extract, filter-sterilized with 0.2µm filter, at the same concentration found in our *C. scoparius*

tissue (0.32 mg alkaloid/1 ml broth). Each of the 12 replicate plates contained 1 well of each treatment per species including two contamination controls. I inoculated each well with a spore or agar slurry created by rinsing cultures with sterile DI H_20 and diluting the spore wash with water in a 1:2 ratio. For non-sporulating species,1 cm^2 pieces of hyphae and 1.5 mL DI water were homogenized with sterile steel beads in a bead beater for one minute. This assay did not include *C.geophilum* or *S. caersulencens*. Cultures grew in ambient light and room temperature conditions for two weeks before harvest. At the end of each assay, fungal biomass was rinsed and collected on pre-weighed filter papers and a buchner funnel. I weighed samples after they dried at 60°C for four days. Three of the 24 contamination control wells showed fungal growth. Plates with contamination were subsequently removed from the study.

For the second assay, the crude alkaloid extract was replaced with pure, filter sterilized (+)-sparteine (TCI America). I used four treatments: a plain PDB control, 1.41 mM (the same sparteine concentration found in the crude extract), 5 mM, and 10 mM. Three 12-well plates together formed one block with each species and two contamination controls randomly assigned to a row of four wells. A row included all four treatments in random order. There were eight replicate blocks.

Statistical analysis: For the crude extract assay, data was analyzed with a two-way ANOVA to see effects of species, treatment, and the interaction of species and treatment on fungal biomass response. Plate number was treated as a random effect.

For the sparteine assay, a two-way ANOVA was also used but plate was not included as a random effect because plate could not be a true block in this design. Because the interaction term was significant, one-way ANOVA was used to test the response of each individual species to sparteine treatments. Data was analyzed with JMP v.11 statistical software (SAS).

Results

Crude alkaloid extract: I recovered 33 mg of alkaloids from 102 g of fresh tissue (0.32mg/g tissue). Analysis of our *C. scoparius* extract by HPLC indicated that it contains only one compound with fragments created by the HPLC-MS apparatus (Fig.1). I identified the unknown alkaloid as sparteine, MW: 234.38 g/mol, using the extract mass spectra (Fig.2).

Bioassays: The crude alkaloid extract significantly decreased fungal biomass overall ($F=3.03$, $df=4$, $p=0.023$ Fig.3). There was a significant difference among species in growth ($F=4.041$,$df=1$,$p=.048$; Fig.3), but I found no significant interaction between alkaloid treatment and species, meaning that species did not differ significantly in their responses to alkaloid addition ($F=0.60$,$df=4$,$p=0.66$; Fig.3). Alkaloid addition also altered the phenotype of *Oidiodendron periconioides* (Fig.4). When grown without alkaloids, the culture was darker than the culture in alkaloids, which appeared yellow.

In the second assay, sparteine concentration significantly affected growth (f=35.62, df=3,3, p <0.0001; Fig. 5). There was also a significant effect of species on growth response (f=4.70, df=6,6, p=0.0002; Fig.5) as well as a significant interaction between sparteine treatment and species (f=5.78, df=18,18, p<0.0001; Fig.5). *C. geophilum* growth significantly decreased with increasing sparteine concentrations (F=16.06, df=3,31,p<0.0001) as did that of *L. fasciculata* (F=8.12, df=3,27, p=0.0005), *L. mutabilis* (F=8.92, df=3,12, p=0.002), *O. periconiodes* (F=21.03, df=3,31, p<0.0001), and *S. caerulescens* (F=6.24, df=3,28, p=0.0022). Sparteine significantly affected *T. lateritia* growth (F=23.43, df=3,28, p<0.0001), but in a non-linear way (Fig. 5). The addition of 1.4mMol and 10 mMol sparteine strongly inhibited growth but 5 mMol sparteine increased growth. There was no effect of sparteine treatment on *W. mikolae* growth (F=0.92, df=3,12, p=0.46).

Discussion

Alkaloid Extraction: The *C. scoparius* crude alkaloid extract yielded approximately 0.32 mg sparteine/g fresh weight, concentrations different from previously reported values. Wink (1981) obtained 0.09 mg alkaloids/g fresh weight in leaves and 0.83 mg alkaloids/g fresh weight in stem tissue and identified 12 different alkaloids, notably lupanine and 17-oxosparteine. While many of the alkaloids were only found in trace amounts (less than 0.02mg/g fresh weight), they were entirely absent from our extraction of *C. scoparius* grown in Washington. Our extraction material was mainly stems, and I intentionally used the same extraction technique as Wink to obtain

comparable concentrations. There are several possible explanations for why I detected less than half the concentration of sparteine measured by Wink. First, stressors in the abiotic environment, like drought, can increase plant alkaloid levels (Höft et al. 1996; Briske et al.1982). Biotic interactions with microbes or herbivores can also stimulate quinolizidine alkaloid production as a defense mechanism in plants (Wink 1987, Hugo et al. 2013). The *Cytisus* used for this study was grown in the greenhouse and probably experienced very little herbivory and water stress, which could account for lower alkaloid levels.

Second, little is known about the origin of the *C. scoparius* used for Wink's extraction. His plants were collected from the field, but the number of individuals and populations sampled in unknown. I utilized tissue from 10 individual young shrubs, from seeds in soil collected from a number of sites. The extent of intraspecific variation of allelochemical production in *Cytisus* is still unknown; but this information is important because for some species, individual populations can share similar alkaloid panels but there can be large differences between populations- even over a short geographical distance (Berkov et al. 2004).

Alternatively, the data suggest the possibility that the chemical panel of *C.scoparius* could be different in its native and invasive range. Our extraction apparently yielded lower levels of sparteine, and fewer alkaloids, than *C.scoparius* growing in western Germany, its native range. These data are consistent with the possibility of reduced

allocation to defense in the new range because of less competitive pressure. Invasive plant species in the introduced range may experience reduced competition compared to their native range so there is less selection for phytochemical production, leading to a decline over time (Lankau et al. 2009).

Nevertheless, these speculations will require additional research on the alkaloid production in both the native and invasive range to ascertain if differences are from the plants and not my methodology or particular sample. As environmental discrepancies between the two regions probably alter intraspecific variation in allelochemicals, a common garden greenhouse study, free of confounding stressors like water and herbivory, might provide the best way of studying the innate alkaloid production of *C. scoparius*.

Bioassays: Both the crude alkaloid extract and pure sparteine affected the growth of fungi. Although the interaction between species and treatment was not significant for the crude extract experiment, a smaller sample size and other differences in the details of the two assays could be responsible for this discrepancy

In general, results from the sparteine assay supported our hypotheses that 1) increasing sparteine concentrations suppress growth and 2) growth responses are species-specific. Growth of most species was significantly suppressed, and sometimes substantially, from 1.4mM sparteine. This is important because both EMF and soil fungi

were affected by even small amounts of sparteine, the amount found in a single gram of *C. scoparius* tissue. The concentrations of *C. scoparius* alkaloids that accumulate in the soil are still unknown, but certainly more than a gram accrues in the soil of invaded forests. This topic warrants future research to determine if the alkaloid levels used in the bioassay are naturally present in the soil and high enough to cause damage to EMF communities in the field.

The data indicate that growth response to sparteine is highly species-specific both across and within functional groups. All of the non-EMF species were negatively affected by sparteine, but there was still a range of severity in growth inhibition (Fig. 5). As for EMF, *C. geophilum* and *S. caerulescens* were very sensitive to sparteine while another, *W. mikolae*, was completely unaffected by even the highest concentrations. *W. mikolae* is known to tolerate poor soil conditions like low pH and heavy metal contamination (Mikola 1988), so this tolerance may also help the species withstand toxic alkaloids. Resistance to sparteine in conjunction with its ability to quickly colonize roots (Mikola 1988) could make it a good choice for restoring *C. scoparius* invaded soils.

Our results are also consistent with Grove et al. (2012) who found a strong decrease in *C. geophilum* colonization of Douglas-fir roots in soils previously invaded by *C. scoparius*. Root colonization was lowered by 50-80% even after the invader's removal. The sensitivity of *C. geophilum* in the field and our assay is especially concerning as it is

a very common Douglas-fir mutalist and present throughout all stages of forest succession (Visser 1995, Goodman et al. 1988) meaning that the decreased colonization and growth of this species may, in turn, have an effect on Douglas-fir restoration.

T. lateritia had unexpected response in which hyphal growth was highest at 5 mM sparteine but lower at 1.4mM and the control. It is possible that a few wells of *T. lateritia* were contaminated with a sparteine tolerant species, which could cause the unusual growth increase at 5 mM. Alternatively, the growth could be the result of a fertilization effect in which low to moderate doses of a chemical actually stimulate growth. de la Vega et al. (1996) found this fertilization response in a similar assay done with sparteine and pathogenic bacteria. They showed that for some species, 15 to 30 mM sparteine actually increased growth, but by 40 mM growth had halted.

Tolerance to sparteine may stem from the ability to decompose alkaloids (Sguros 1955) possibly to use the byproducts as a source of carbon and nitrogen (Shukla 1986, Hakil et al. 1998). Fungal species capable of utilizing nutrients from toxic alkaloids may then flourish in the presence of *C.scoparius*. This could possibly shift community composition in the soil from sensitive to tolerant species in the month-long timeframe sparteine takes to fully decompose in the soil (Gross and Wink, 1986). Based on EMF response to sparteine in our assay, I would expect to see dominance by *W. mikolae* in *Cytisus* rich sites while *T. lateritia* could also be a prominent EMF in lower sparteine concentrations.

This study provides the first direct look at how EMF respond to the allelopathic alkaloids of *C. scoparius*. While species' response to sparteine varied, the inhibition of growth by sparteine for most species suggests that allelochemicals from *C. scoparius* could pose a problem to Douglas-fir trees through their native fungal communities. Further research studying additional EMF as well as their responses in the field will provide a more complete picture of how the allelochemicals of *C. scoparius* may be assisting invasion. Such research is essential for restoring damaged fungal communities and may aid in the eventual reestablishment of native flora.

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Figures

Species	Family	Division	Ecological Role
Non-EMF			
Lecythophora fasciculata	Coniochaetaceae	Ascomycota	Possible root endophyte
Lecythophora mutabilis	Coniochaetaceae	Ascomycota	Root endophyte, wood-rot
Oidiodendron periconioides	Myxotrichaceae	Ascomycota	Ericoid mycorrhiza
EMF			
Cenococcum geophilum	Gloniaceae	Ascomycota	
Suillus caerulescens	Suillaceae	Basidiomycota	
Tomentella lateritia	Thelephoraceae	Basidiomycota	
Wilcoxina mikolae var. tetraspora	Pyronemataceae	Ascomycota	

Table 1. Douglas-fir EMF and associated fungi isolated from roots for use in bioassay.

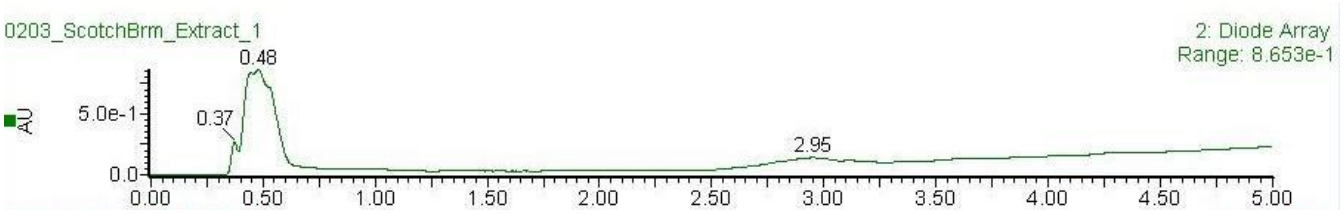

Figure 1. HPLC-UV chromatogram of *C. scoparius* crude alkaloid extract. At 0.37 and 0.48 minutes are peaks for sparteine fragments and sparteine respectively.

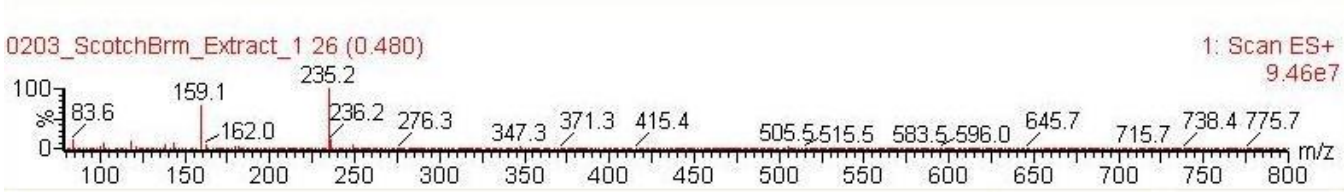

Figure 2. Mass spectra of *C. scoparius* crude alkaloid extract. Peaks at 83.6 correspond to solvent, and 159.1 and 235.2 to sparteine and sparteine fragments.

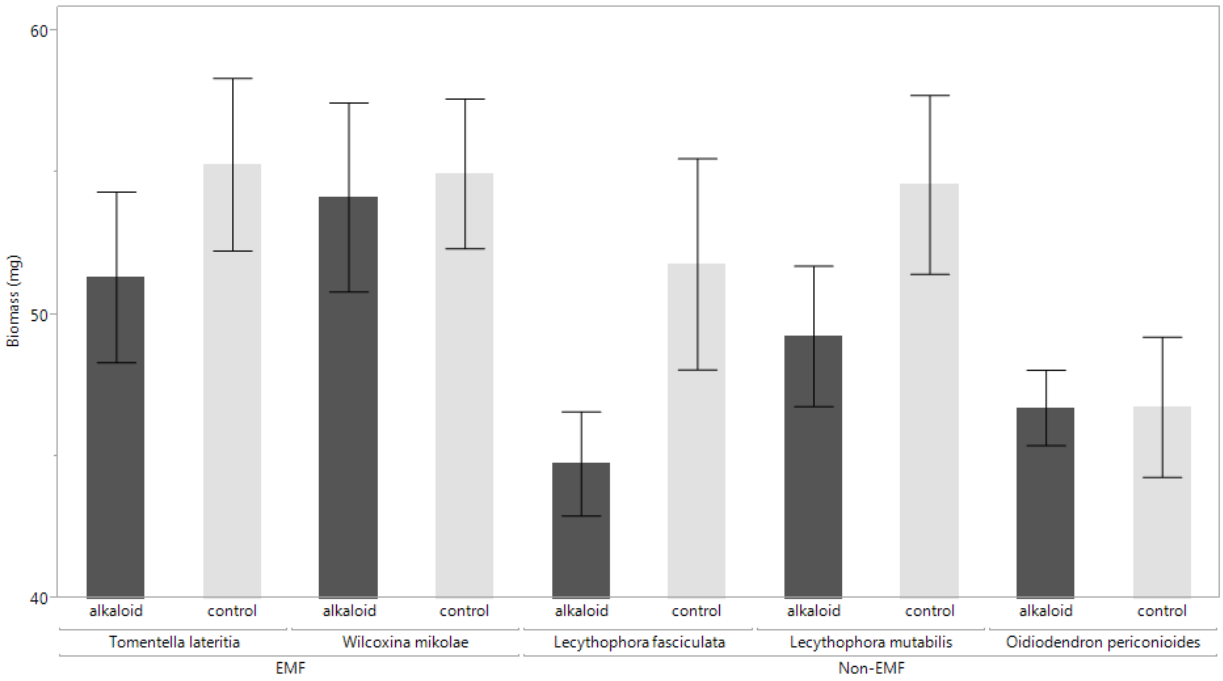

Figure 3. Effect of crude alkaloid extract treatment on growth of five Douglas-fir associated fungal species after 14 days. Error bars represent ±1SE.

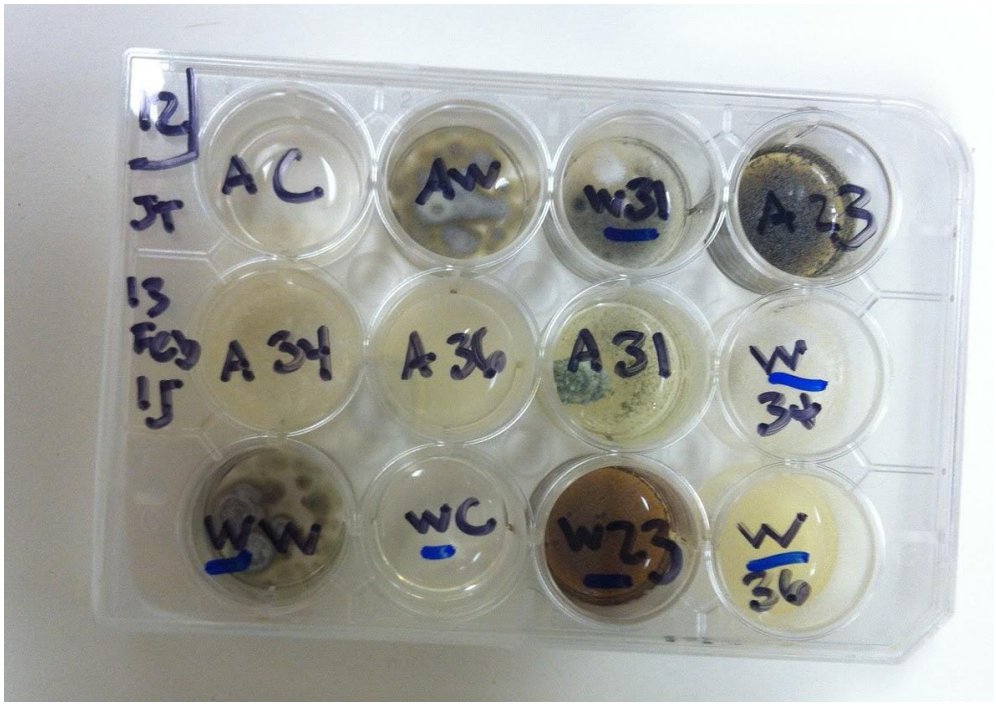

Figure 4. 12-well plate of *C. scoparius* crude alkaloid extract assay. Alkaloid treatment was marked with ‘A’ and the control with ‘W’. Species are labeled numerically or with another ‘W’. *Oidiodendron periconioides* was species ‘23’.

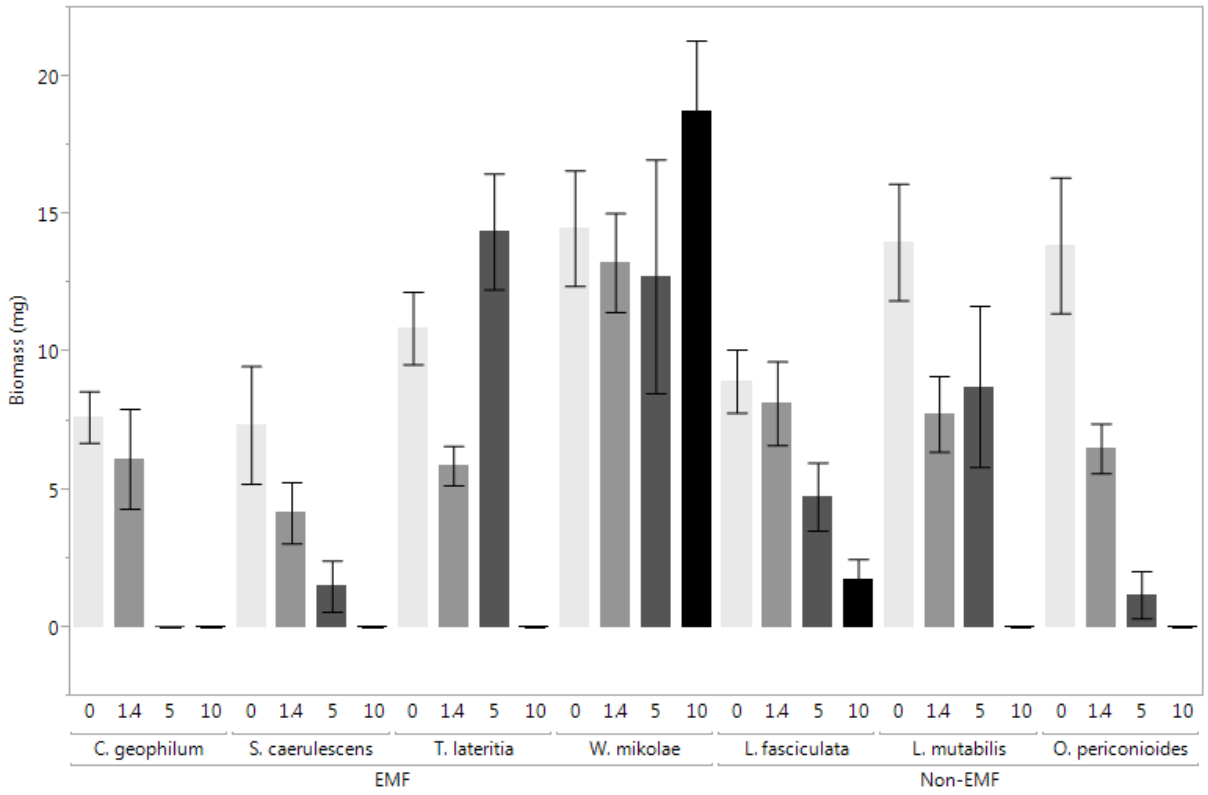

Figure 5. Growth response of Douglas-fir associated fungi to increasing concentrations of sparteine after 14 days. Numerical values on x-axis show sparteine concentrations in mM. Error bars represent ±1SE.